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Abstracts of Poster Session

Lack of Mutagenicity of Baghouse Samples in a Modified Ames Plate Assay. C. WITMER and K. COOPER, *Rutgers University, Piscataway, NJ.*

A modified Ames system was used to test a series of unknown compounds initially identified only as inorganic oxides and baghouse samples. Later the samples were identified as three iron oxides (Fe_3O_4, gamma Fe_2O_3), a mixture of 50% chromic chromate (CrO_3/Cr_2O_3, 1:2) and 50% alpha Fe_2O_3, and seven baghouse samples. In order to better detect inorganic compounds as mutagens in the Ames test, the concentrations of all salts were lowered (1/3, 1/2, 2/3 and 3/3 the normal Ames concentration) and the time for mutagenicity using strains TA 100 and TA 1535 at concentrations up to 2.0 mg/plate. The iron oxides were not found to be mutagenic or toxic at these concentrations. Studies with pure chromic chromate indicated that the optimum concentration of salts for scoring both mutagenicity and toxicity varied depending on mutagen concentration and bacterial strain. Chromic chromate when tested alone or in the iron oxide mixture was mutagenic only at toxic levels. With strains TA 1535 and TA 100, the chromic chromate mixture (18% chromium) at a concentration of 1.0 mg/plate was found to be mutagenic. With strain TA 1535, increased mutagenicity was also obtained as salt concentration was decreased after 48 and 72 hours of growth. Spectrophotometric analysis determined that five of the baghouse samples contained 0.1% chromium. Two samples were composed of 0.2% chromium and 0.6% chromium, respectively. Only one sample had benzene-soluble fraction total particulate matter (BSFTPM) of 1.8%. The other six samples had a BSFTPM of 0.1% or less. All seven baghouse samples were found to be non-mutagenic and nontoxic to the bacteria.

Thymidine Incorporation by Lung Fibroblasts as a Sensitive Assay for Biological Activity of Asbestos. I. LEMAIRE, G. GINGRAS and S. LEMAIRE, *Unite de Recherche Pulmonaire, Faculté de Medecine, Université de Sherbrooke, Sherbrooke, Quebec J1H 5N4, Canada.*

Asbestos fibers reach the connective tissue compartment of the lung, and it is likely that direct interaction of fibers with the pulmonary interstitium may have determinant effects in asbestos-related diseases. In order to define a parameter in fibroblast function which may be related to the biological activity of asbestos, we investigated the effects of asbestos and various particles on *in vitro* DNA synthesis and growth of lung fibroblasts. Fibroblasts exposed to chrysotile B displayed an initial growth inhibition compared to control during the first 24 hr of incubation. At 48 and 72 hr, however, there was resumption of cell proliferation as evidenced by elevated thymidine incorporation and cell counts. UICC samples of chrysotile A, chrysotile B, crocidolite, amosite and anthophyllite all altered thymidine incorporation by lung fibroblasts in a dose-dependent manner and to different maximal levels. Amphibole asbestos were either equally or less active than chrysotile. Silica had the lowest activity. Various other particles including latex, zymosan, titanium dioxide, magnetite and Mt. St. Helens volcanic ash had no effect. The DNA synthesis reponse of human lung fibroblasts to asbestos correlates with its *in vivo* fibrogenic and tumorigenic potential and may prove useful in assessing rapidly the biological activity of asbestos and related compounds.

Modulation of the *in Vitro* Reactivity of Pulmonary Macrophages to Chrysotile Asbestos as a Function of Time in Culture.

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When the cytotoxic effects of chrysotile (Ch) asbestos on freshly isolated (FI) macrophages (MØ) and on MØ kept in monolayer (ML) for various periods of time were compared, it was found that the pattern of extracellular enzyme release was altered. With FIMØ, the primary toxicity (LDH release) was usually higher than the secondary toxicity (α-galactosidase, or α-GAL, release). For MLMØ, the opposite pattern was usually observed. A time-course study showed that the longer the MLMØ stayed in culture before being challenged by Ch fibers, the more selective was the enzyme leakage pattern. While the percentage of release of α-GAL by MLMØ following Ch exposure stabilized after 24 hr of preincubation, the LDH leakage progressively decreased to control levels after 72 hr of preincubation. By comparison, no such changes were observed with the positive control dust DQ12: the LDH release was always greater than the α-GAL release. Only the dose-response curve was shifted to the right when MLMØ preincubated for 24 hr were compared to FIMØ.

The much more rapid decrease in LDH release over preincubation time by MLMØ following Ch exposure is indicative that the plasma membrane is profoundly altered by the adherence process of MØ to artificial substrates used in cell culture. The significance of this phenomenon in relation with the *in vitro* cytotoxicity of Ch asbestos fibers is discussed.

Biological Evaluation of Various Natural and Man-Made Mineral Fibers: Cytotoxicity, Hemolytic Activity and Chemilunescence Study.

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The biological effects of eleven natural and man-made mineral fibers were compared with four UICC asbestos standards. Cytotoxicity (C) of the fibers for rat pulmonary alveolar macrophages (MØ), hemolytic activity (HA) for rat erythrocytes and activating effect on oxidant production (OX) in rat MØ were used to compare the biological reactivity *in vitro*. It was found that for samples of wollastonite, mineral wool (type 1 and 2), glass fiber, kaowool and gypsum, they were more or less unreactive as far as CMØ and HA were concerned. OX in rat MØ was nonetheless significant for wollastonite, gypsum, type 2 mineral wool (D_f = 3.3 μm; L_f = 221.4 μm) and kaowool fibers. As reflected by their HA, mineral fibers like attapulgite and micro glass fiber (D_f = 0.2 μm; L_f = 11.4 μm) were highly reactive: attapulgite was as hemolytic as the UICC chrysotile B (Ch) standard, followed by micro glass fiber, sepiolite and erionite. The HA of the last two samples were lower than the HA of Ch, but were nonetheless higher than the UICC amphiboles standards (crocidolite < amosite < anthophyllite).

For the CMØ, dose-response relationships were observed with all asbestos samples, as well as for attapulgite, micro glass fiber, sepiolite and erionite fibers. Primary and secondary toxicity were evaluated, respectively, by the extracellular release of LDH (cytoplasmic) and α-galactosidase (lysosomal) enzymes. It was observed that only the Ch sample produced a parallel enzyme leakage (LDH/α-GAL ratio > 1.0). On the other hand, erionite, micro glass fiber, amphibole asbestos, attapulgite and sepeolite produced a more selective leakage, that is, a LDH/α-GAL ratio > 1.5. All cytotoxic events were confirmed by the reduction of cellular ATP and a decrease in production of lactic acid. Attapulgite fibers induced the strongest OX response, followed by crocidolite, anthophyllite, sepiolite, amosite, Ch and erionite fibers. Although it was difficult to establish a clear cut relationship between the biological reactivity and the number of fibers for all type minerals, it seems that for nonasbestos samples, the *in vitro* biological effects were somewhat related with the number of fibers present in the samples. As far as asbestos samples are concerned, and especially for Ch, chemical surface properties might also be involved.

In conclusion, despite the fact that the relative biological potency of mineral fibers *in vitro* can be achieved on a dose-response basis, absolute comparison cannot be achieved unless samples of comparable aspect ratios can be made available to investigators.

Quantification of Interactions Between Cigarette Smoke and Industrial Contaminants.

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An elevated cancer risk for human populations is often associated with their exposure to multiple factors, e.g., cigarette smoke and asbestos. A model system was designed to study such interactions between multiple environmental factors in order to indicate which combinations of these factors would result in additive, synergistic or antagonistic effects on response patterns. The study focuses on the quantification of genotoxic effects in cell cultures exposed to combinations of cigarette smoke and a variety of compounds of industrial importance: asbestos, arsenic, chromium, nickel, benzene and formaldehyde. Two genotoxic assays are employed: the induction of chromosome aberrations in Chinese hamster ovary (CHO) cells and of gene conversions in the yeast *Saccharomyces cerevisiae* strain D7. The test cells are exposed to cigarette smoke produced by a "cigarette inhalation machine," which can simulate a variety of human exposure patterns, e.g., a "nervous fast" smoker or a "relaxed slow" smoker. Three to six puffs of cigarette smoke will induce chromatid breaks and exchanges in exposed CHO cells and gene convertants in the yeast. To study multiple factor interactions, the test cells can be exposed to various agents either prior to, during or after the exposure of the cells to the cigarette smoke. Preliminary studies indicate that some tested combinations result in a synergistic effect on the genotoxic activities studied, e.g., cigarette smoke and the transition metal Cu^{2+}, cigarette smoke and chromate (K_2CrO_4). In contrast, exposure of the test cells to reducing agents (ascorbate, cysteine, glutathione) prior to the cigarette smoke causes a reduction in the observed genotoxicity. These studies are currently being extended to the other environmental contaminants indicated.

Binding of Environmental Carcinogens to Asbestos and Mineral Fibers.

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The incidence of bronchial or pulmonary carcinoma is statistically higher among asbestos workers who smoke. It is possible that asbestos might not be a true carcinogen but a promoter due to its binding capacity for carcinogens present in the environment or in tobacco smoke. We have developed a rapid test for measuring the carcinogen binding capacity of asbestos and other mineral fibers. Benzopyrene (BaP), nitrosonornicotine (NNN), and *N*-acetyl aminofluorene (NAAF) were assayed in the presence of crude 4T30 Canadian asbestos, UICC chrysotile, amosite, crocidolite, glasswool, glass fiber and titanic oxide. A constant amount of each test material was incubated with tritium-labeled carcinogens, washed and counted. The binding of carcinogens to all asbestos fibers was significantly higher than binding to glass fiber and to titanic oxide. The influence of pH on the binding was also studied. The *in vitro* cytotoxicity on P388D1 cells and the hemolytic activity on various mineral fibers were also measured. A correlation factor of 0.82 was found between the benzopyrene binding, the cytotoxicity and the carcinogenicity of the fibers. These tests will be included in a series of short-term assays to evaluate the carcinogenicity of natural and modified fibers.

Association of DNA Degradation in Cultured Human Fibroblasts with the Cytotoxicity of Fibrous Substances.

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Human fibroblasts incubated with (^{3}H)TdR to generally label cellular DNA released radioactivity into culture medium after exposure to UICC chrysotile B asbestos. This release was initially thought to reflect DNA degradation in viable cells, because trypan blue staining revealed low numbers of nonviable cells. But the low number of nonviable cells was due to their reduced staining in medium containing 15% serum. Under more appropriate conditions, 40-60% nonviable cells were observed in four different viability assays after exposure to 100 μg/mL UICC chrysotile B for 96 hr. The release of radioactivity was associated with reduced numbers of attached cells, but not the sensitization of cells to UICC chrysolite B by ^{3}H decay-induced damage to the nucleus. These results suggest the release of radioactivity is not due to DNA degradation in viable cells or an artifact of radiolabeling, but rather the cytotoxicity of UICC chrysotile B asbestos.

No release of radioactivity was observed after exposure to 100 μg/mL of codes 100, 108, and 110 glass fiber, silica, volcanic ash, and UICC amosite, anthophyllite, and crocidolite for 96 hr. Release was observed with UICC chrysotile A, hand-milled code 100 glass fiber, and UICC chrysotile B. The active samples had the smaller fiber diameters of the samples tested, and the percent length distribution of the milled code 100 glass fiber was similar to that of the UICC chrysotile B. Cytotoxicity may therefore be related more to fiber size than composition.

Morphologic alterations induced by UICC chrysotile B in toluidine blue-stained cells were evident in the continuity of the plasma and nuclear membranes and the intensity and pattern of chromatin staining. Each of the above samples at 0.1, 1, 10 and 100 μg/mL were examined for their ability to produce alterations. Alterations were produced by substances which did not induce a release of radioactivity, and by the biologically active samples at lower concentrations than those required for the release of radioactivity. But the only major and dose dependent alterations were produced by samples which induced the release of radioactivity at higher concentrations. The results suggest the morphologic alterations are an early sign of the cytotoxicity of fibrous substances, and that the release of radioactivity may be of use in the preliminary screening of fibrous substances for cytotoxicity.

A Preliminary Ultrastructural Study of Macrophage Response to Carbon Fiber Particles.

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Mouse peritoneal macrophages stimulated with thyoglycolate broth medium were examined ultrastructurally for their reaction to IP injections of carbon fiber particles (less than 40 μm long and 7-8 μm in diameter). The interaction of carbon particles with macrophages was compared to responses generated by asbestos (chrysotile—diameters < 5 μm and varying lengths) and by glass fiber (< 40 μm in length and 5-8 μm in diameter). Samples of stimulated macrophages were plated at 24, 48, 120, and 144 hr and the attached cells were prepared for SEM. Companion samples were also prepared for TEM. Phagocytic activity appeared to progress in the same manner, regardless of fiber size or content. Cell adhesion occurred initially at the attachment involving multiple cells. Asbestos activity usually involved single cell encapsulation of fibers due to a small fiber size. Multiple cell responses were generated in both fiberglass and carbon particles, primarily due to the greater diameter of these particles. As shown in the 120- and 144-hr samples, complete coverage of these large particles occurred, forming a mini-encapsulation cyst. TEM examination was used to compare membrane encapsulation of all three fiber types. Carbon particles generated comparable phagocytic response as asbestos, a known hazardous material. With this in mind, this preliminary study has indicated a need to pursue the role carbon particles play as potential environmental contaminants for human respiratory concern.